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Reviewed RDB 10/7/03

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Date: June 16, 2003

Mr. Steve Rosenbaum
California Regional Water Quality Control Board -
Central Valley Region
3443 Routier Road, Suite A
Sacramento, CA 95827-3003

Dear Mr. Rosenbaum:

Please find attached the results of the first installment of the in-stream biotoxicity assessment monitoring required by Waste Discharge Requirements Order No. 5-00-028 for the U.S. Department of Agriculture, Forest Service, Plumas National Forest at the Walker Mine Tailings in Plumas County. This report is for samples collected November 3-15, 2002. Macroinvertebrate samples were analyzed by the National Aquatic Monitoring Center at Utah State University. Periphyton samples were analyzed by Hannaea laboratory in Helena, Montana.

The 2001 Amended Record of Decision for the Walker Mine Tailings site provides for the diversion of Dolly Creek around the tailings material. A contract has been awarded to Ecology and Environment, Inc. of San Francisco for the design of this diversion channel and the final design is due to be delivered to the Plumas National Forest later this summer. Negotiations with the Atlantic Richfield Company (ARCO), a Potentially Responsible Party, over remediation costs are continuing.

Please call Joe Hoffman of this office at (530) 283-7868 if you have questions.

I CERTIFY UNDER PENALTY OF LAW THAT I HAVE PERSONALLY EXAMINED AND AM FAMILIAR WITH THE INFORMATION SUBMITTED IN THE ATTACHED DOCUMENTS AND THAT, BASED ON MY INQUIRY OF THOSE INDIVIDUALS IMMEDIATELY RESPONSIBLE FOR OBTAINING THE INFORMATION, I BELIEVE THAT THE INFORMATION IS TRUE, ACCURATE, AND COMPLETE. I AM AWARE THAT THERE ARE SIGNIFICANT PENALTIES FOR SUBMITTING FALSE INFORMATION, INCLUDING THE POSSIBILITY OF FINE AND IMPRISONMENT.

Sincerely,

for JAMES M. PEÑA
Forest Supervisor

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BIOLOGICAL MONITORING REPORT

WDR Order Number: 5-00-028

Discharger: USDA Forest Service, Plumas National Forest

Facility: Walker Mine Tailings, Plumas County

Reporting Frequency: Annual

Monitoring Period: 2002

Findings:

Macroinvertebrate and periphyton samples from Little Grizzly Creek and Indian Creek were collected November 3-15, 2002. Sample collection was performed per the U.S. Forest Service's Pacific Southwest Region Stream Bioassessment Protocols and was consistent with the in-stream biotoxicity assessment program for Walker Mine Tailings that was approved by the CA Regional Water Quality Control Board in March 2002.

The macroinvertebrate and periphyton sample analyses both indicate significant aquatic health impairment at site 2, the first site on Little Grizzly Creek that is situated below the confluence of Little Grizzly Creek and the stream that flows over the mine tailings, Dolly Creek (site 2 is located at Brown's Cabin, which is the WDR compliance station for chemical and physical water quality). The analyses for both components (macroinvertebrate and periphyton) demonstrated full recovery from the tailings pollution at site 6, the lowest sample site on Little Grizzly Creek situated approximately 8 miles downstream of the confluence with Dolly Creek (see the attached map). Sample results demonstrated no measurable impact between sites 7 and 8 on Indian Creek, indicating that the mine tailings pollution in Little Grizzly Creek is not affecting Indian Creek.

Site 1 is located on Little Grizzly Creek above its confluence with Dolly Creek and was intended to be used as a background sample location. While it would be expected that the background site analyses would indicate the highest levels of water quality, some indications of impairment can be observed in the macroinvertebrate data for site 1. Of the 8 sites surveyed, site 1 ranked just fifth in diversity (Shannon) and fourth in taxa richness (total) (see Table 1). Additionally, the dominant family observed at site 1 is the Chironomidae (midge) family, a group of macroinvertebrates that are decidedly tolerant of poor water quality. The periphyton data indicates good water quality except for moderate impairment due to siltation (sedimentation); this impairment is indicated by the elevated concentration of motile periphyton species observed at site 1. Site 1 is situated just downstream of a 2-mile long meadow reach of Little Grizzly Creek. This is the only reach of Little Grizzly Creek that is subjected to cattle grazing; trampled and eroding banks, as well as organic input from cattle feces, are possible causes for the moderate water quality impairments indicated at site 1.

While the biological sample results for site 1 do not demonstrate pristine water quality, the results do provide a solid background for comparison with site 2, which is situated just downstream of the confluence with Dolly Creek. The analyses for both biological components indicate a significant decrease in water quality from site 1 to site 2. The macroinvertebrate results rank site 2 as the poorest of the 8 sites surveyed for nearly all parameters, including abundance of organisms, richness, diversity, and Hilsenhoff Biotic Index. As with site 1, Chironomidae represented the dominant family, but this dominance was more pronounced at site 2 with Chironomidae making up 57% of the organisms observed (up from 31% at site 1). Similarly, the periphyton results indicated low diatom species richness and diversity. The diatom species observed were dominated by *Achnanthidium minutissimum* (77%), a species known to be tolerant of acid mine drainage and the associated elevated concentrations of heavy metals (see Table 5 of the attached periphyton report).

Both biological components indicate steady recovery for the aquatic health of Little Grizzly Creek as it flows downstream from its confluence with Dolly Creek; this recovery is virtually total and complete at site 6. Macroinvertebrate richness and diversity increase steadily from sites 3 to 5 and, for the 8 sites surveyed, rank highest at site 6. Periphyton diatom metrics demonstrate good biological integrity with only minor impairment from sedimentation and heavy metals. Across the board for the 8 sites surveyed, site 6 appears to provide the most pristine water quality for aquatic organisms. Little Grizzly Creek above site 6 is a stable, canyon reach fed by pristine, unroaded tributary streams. Small-scale recreational mine dredging is the only significant disturbance along this reach and site 6 is situated well downstream of the above mentioned impairments due to cattle grazing and mine tailings pollution.

The biological analyses results demonstrate that flow from Little Grizzly Creek – and subsequently the mine tailings pollution found in Dolly Creek – is not impacting the aquatic health of Indian Creek. Macroinvertebrate richness and diversity, as well as the Hilsenhoff Biotic Index, are remarkably similar for the two Indian Creek sites (7 and 8). The dominant family observed at the two sites is the sensitive Hydropsychidae family, an indication of good water quality. Periphyton analyses on the two Indian Creek samples indicate good biological integrity with only minor impairment from sedimentation.

Given that the biological water quality parameters for Little Grizzly Creek at site 6 - just upstream of its confluence with Indian Creek - were the best for any of the sites surveyed, it is logical that any measurable effect from the flow of Little Grizzly would actually be an improvement in Indian Creek water quality. This trend is supported by the observed increase in macroinvertebrate species abundance from site 7 to site 8. If these trends continue for the next year or two of biological monitoring, serious consideration should be given to eliminating sites 7 and 8 from future studies as it is unlikely that such monitoring will demonstrate any measurable impacts on Indian Creek due to pollution from the Walker mine tailings site.

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Location Descriptions: Walker Mine Tailings Biological Sampling Sites

On Little Grizzly Creek:

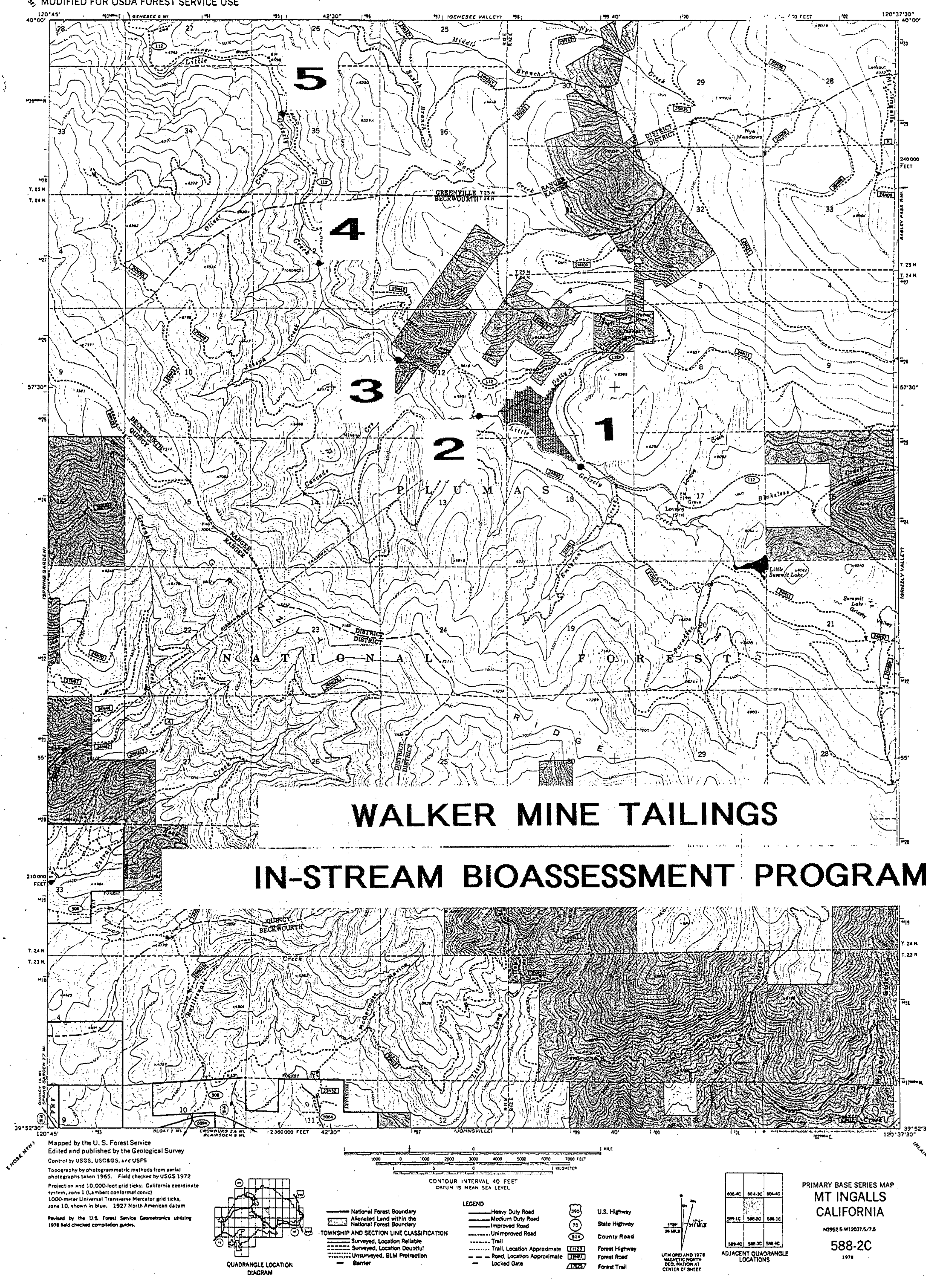
1. At surface water monitoring site R-3, Little Grizzly Creek upstream of the tailings area. The site will be used as a control.
2. At surface water monitoring site R-5, Little Grizzly Creek downstream of its confluence with Dolly Creek and the tailings area and upstream of the Brown's Cabin spring. R-5 is the surface water compliance station.
3. Immediately downstream from Cascade Creek.
4. Approximately 1100 feet downstream from Joseph Creek and immediately downstream from an unnamed stream.
5. Approximately 1700 feet downstream from Oliver Creek.
6. At the USGS gage site (no longer operated), approximately one mile upstream from Genessee Valley.

On Indian Creek:

7. Upstream of Little Grizzly Creek near County Road 112 bridge. The site will be used as a control site.
8. Approximately 2000 feet downstream from Little Grizzly Creek.

UNITED STATES
DEPARTMENT OF THE INTERIOR
GEOLOGICAL SURVEY
MODIFIED FOR USDA FOREST SERVICE USE

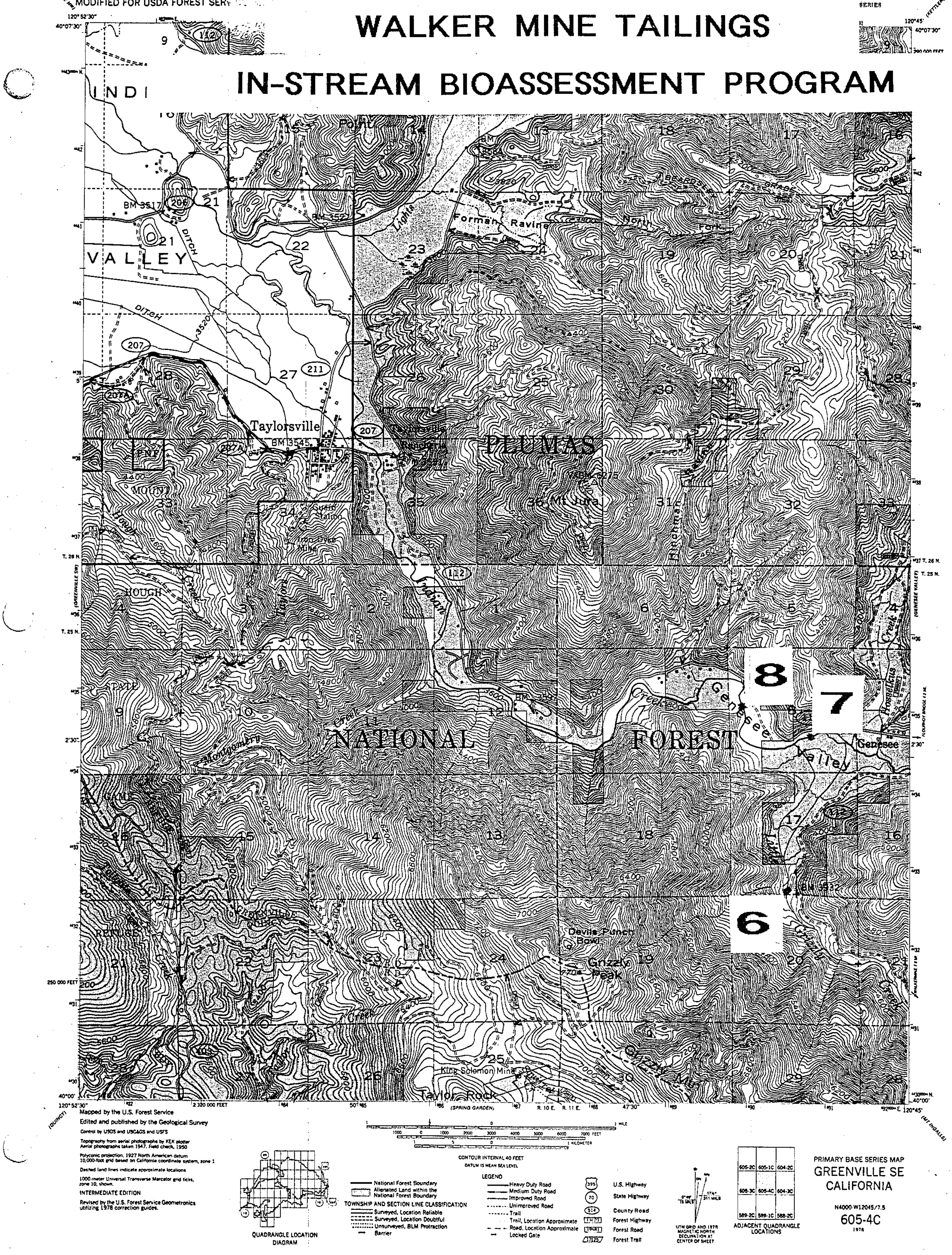
SAMPLE SITE LOCATION MAP



SAMPLE SITE LOCATION MAP

WALKER MINE TAILINGS

IN-STREAM BIOASSESSMENT PROGRAM



BIOASSESSMENT 2002

AQUATIC MACROINVERTEBRATE ANALYSES REPOT

TABLE 1: MACROINVERTEBRATE RESULTS SUMMARY

IN-STREAM BIOTOXICITY ASSESSMENT PROGRAM NO. 5-00-028
U.S. DEPARTMENT OF AGRICULTURE, PLUMAS NATIONAL FOREST
WALKER MINE TAILINGS, PLUMAS COUNTY
NOVEMBER 2002

Sample Station	Total Abundance (rank)	EPT* Abundance (rank)	Dominant Family (% contributed)	Total Taxa Richness (rank)	Shannon Diversity (rank)	Hilsenhoff Biotic Index (rank)
1	4194 (4)	1366 (6)	Chironimidae (31%)	35 (4)	2.57 (5)	3.84 (6)
2	198 (8)	41 (8)	Chironimidae (57%)	15 (8)	1.59 (8)	4.71 (8)
3	5463 (2)	1941 (4)	Chironimidae (56%)	26 (7)	2.20 (7)	4.37 (7)
4	5591 (1)	2867 (2)	Chironimidae (41%)	35 (3)	2.43 (6)	3.59 (4)
5	5241 (3)	4407 (1)	Hydropsychidae (26%)	41 (2)	2.77 (2)	2.68 (1)
6	2054 (6)	1633 (5)	Heptageniidae (21%)	42 (1)	3.06 (1)	2.90 (2)
7	1512 (7)	1072 (7)	Hydropsychidae (37%)	31 (6)	2.72 (4)	3.44 (3)
8	2723 (5)	2123 (3)	Hydropsychidae (41%)	34 (5)	2.73 (3)	3.72 (5)

Aquatic Macroinvertebrate Monitoring Report

Report Prepared for:
U.S. Forest Service
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Report Prepared by:
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U.S.D.I. Bureau of Land Management
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24 April 2003

Sampling Methods

Field sampling dates and aquatic invertebrate collection methods for each sample. In station descriptor, QL = qualitative sample.

Sample ID	Station	Date	Sampling Method	Habitat Sampled	Sampling Area (m2)	Comments
119869	PNFWALKER1	11/03/2002	Kick net	Riffle	1.000	None
119870	PNFWALKER2	11/03/2002	Kick net	Riffle	1.000	None
119871	PNFWALKER3	11/04/2002	Kick net	Riffle	1.000	None
119872	PNFWALKER4	11/06/2002	Kick net	Riffle	1.000	None
119873	PNFWALKER5	11/09/2002	Kick net	Riffle	1.000	None
119874	PNFWALKER6	11/11/2002	Kick net	Riffle	1.000	None
119875	PNFWALKER7	11/15/2002	Kick net	Riffle	1.000	None
119876	PNFWALKER8	11/14/2002	Kick net	Riffle	1.000	None

Laboratory Methods

Samples were identified at the Western Bioassessment Center, Logan, Utah. The basic procedures we followed for processing the samples are described in Cuffney et al. 1993 (Methods for collecting benthic invertebrate samples as part of the National Water-Quality Assessment Program. United States Geological Survey Open-File Report 93-406) and are described in greater detail and are rationalized in Vinson and Hawkins 1996 (Effects of sampling area and subsampling procedures on comparisons of taxa richness among streams. Journal of the North American Benthological Society 15:393-400).

If the sample appeared to contain more than 500 organisms, it was sub-sampled. Sub-samples were obtained by pouring the sample into an appropriate diameter 250 micron sieve, floating the material by placing the sieve within an enamel pan partially filled with water and leveling the material within the sieve. The sieve was then removed from the water pan and the material within the sieve was divided into equal parts. This process was repeated until it appeared that approximately 500 organisms remained in one-half of the sieve. This material was then placed little-by-little into a petri dish and all organisms were removed under a disecting microscope at 10-60 power. Additional sub-samples were taken until at least 500 organisms were found. Once a sub-sample was started, all organisms within it were removed. The total number of organisms removed and retained from each sample is listed below. When the sorting of the sample splits was completed, the entire sample was spread throughout a large white enamel pan. The entire sample was then searched for 10 minutes to remove any taxa that might not have been picked up during the intial sample sorting process. The objective of this "big/rare" search was to provide a more complete taxa list by finding rarer taxa that may have been excluded during the sub-sampling process. All the organisms removed during the sorting process were then identified by well-qualified taxonomists. An effort was made to identify organisms to a consistent taxonomic level. Insects were primarily identified to genus, with the exception of Chironomidae which were identified to subfamily. Non-insect invertebrates were identified to various taxonomic levels depending on the availability of identification keys. Voucher specimens were retained for all unique taxa. The identified portion of the sample was placed in 70% ethanol, given a unique catalog number, and will be kept forever, unless invertebrate dry weights were determined.

Laboratory sample processing information. The percentage of each sample processed and the total number of invertebrates identified for each sample is reported. In station descriptor, QL = qualitative sample.

Sample ID	Station	Date	Field split %	Lab split %	% id'd	Invertebrates identified	Comments
119869	PNFWALKER1	11/03/2002	None	13	13	533	None
119870	PNFWALKER2	11/03/2002	None	None	100	198	None
119871	PNFWALKER3	11/04/2002	None	9	9	526	None
119872	PNFWALKER4	11/06/2002	None	9	9	538	None
119873	PNFWALKER5	11/09/2002	None	13	13	670	None
119874	PNFWALKER6	11/11/2002	None	25	25	530	None
119875	PNFWALKER7	11/15/2002	None	38	38	574	None
119876	PNFWALKER8	11/14/2002	None	19	19	526	None

Data analysis and interpretation

This section is provided as an introduction to interpreting aquatic macroinvertebrate sample results. Additional information can be found in the resources cited at the end of this section. A variety of data measures have been developed to assess the stream health using aquatic macroinvertebrates. Those most commonly used are described below and have been calculated for your samples.

Benthic macroinvertebrates are important elements of water quality evaluations because they (1) live in, on, or near streambed sediments; (2) have relatively long life cycles; and (3) are relatively sessile compared with larger organisms, such as fish. This combination of characteristics ensures that benthic invertebrates (1) respond to natural and anthropogenic environmental conditions that physical or chemically alter streambed sediments; (2) integrate effects over a year; and (3) characterize effects over a relatively small spatial scale (in contrast with fish, which may travel long distances). These factors make benthic invertebrates well suited for use in assessing site-specific water quality and comparing spatial patterns of water quality at multiple sites, and for integrating effects up to a year after a pollution or disturbance event.

The occurrence of benthic invertebrates in a stream is a response to natural and anthropogenic influences. Rivers naturally change as they flow downstream. Riparian vegetation conditions, light, temperature, hydraulics, and substrate composition all change and in response to these environmental changes benthic invertebrate communities change. Thus, each location in a river has a range of environmental conditions that dictate which invertebrate species are found there.

Taxa richness - Richness is a component and estimate of community structure and stream health based on the number of distinct taxa. Taxa richness normally decreases with decreasing water quality. In some situations organic enrichment can cause an increase in the number of pollution tolerant taxa.

Abundance - The abundance, density, or number of aquatic macroinvertebrates per unit area is an indicator of habitat availability and fish food abundance. Abundance may be reduced or increased depending on the type of impact or pollutant. Increased organic enrichment typically causes large increases in abundance of pollution tolerant taxa. High flows, increases in fine sediment, or the presence of toxic substances normally cause a decrease in invertebrate abundance.

EPT - A summary of the taxa richness and abundance among the insect Orders Ephemeroptera, Plecoptera, and Trichoptera (EPT). These orders are commonly considered sensitive to pollution.

Number of families - All families are separated and counted. The number of families normally decreases with decreasing water quality.

Percent taxon or family dominance - A community dominated by a single taxon or several taxa from the same family suggests environmental stress.

Shannon Diversity Index - Ecological diversity is a measure of community structure defined by the relationship between the number of distinct taxa and their relative abundances.

$$H = -\sum_{i=1}^{S} (p_i \ln p_i) \quad (1)$$

The Shannon index has been the most widely used in community ecology. The Shannon index was calculated as:

where H is the index of species diversity, S is the total number of taxa, and p_i is the proportional abundance of the *i*th species. The higher the number the greater the diversity. Another commonly used diversity index is Simpson's Index of diversity. The Simpson Index was calculated as:

$$D = 1 - \sum_{i=1}^{S} (p_i)^2 \quad (2)$$

where D is the Simpson index of diversity and p_i is the proportion of individuals of taxa *i* in the assemblage. Simpson's index gives little weight to the rare taxa and more weight to the common taxa. It ranges in value from 0 (low diversity) to a maximum of (1 - 1/*S*), where *S* is the number of taxa.

Evenness - Evenness is a measure of the distribution of taxa within a community. The evenness index used in this report was calculated as:

$$Evenness = \frac{(1/\lambda) - 1}{(e^{H}) - 1} \quad (3)$$

where,

$$\lambda = \sum_{i=1}^{S} (p_i^2) \qquad (4)$$

H is Shannon's diversity index (Equation 1) and S is the total number of taxa, and p_i is the proportional abundance of the *i*th species. Value ranges from 0-1 and approach zero as a single taxa becomes more dominant.

Biotic indices - Biotic indices use the indicator taxa concept. Taxa are assigned water quality tolerance values based on their specific tolerances to pollution. Scores are typically weighted by taxa relative abundance. In the United States the most common biotic indices in use are the Hilsenhoff Biotic Index and the USFS Biotic Condition Index that has been principally used by the Bureau of Land Management and the United States Forest Service.

Hilsenhoff Biotic Index - The Hilsenhoff Biotic Index (HBI) summarizes the overall pollution tolerances of the taxa collected. This index has been used to detect nutrient enrichment, high sediment loads, low dissolved oxygen, and thermal impacts. It is best at detecting organic pollution. Families were assigned an index value from 0- taxa normally found only in high quality unpolluted water, to 10- taxa found only in severely polluted waters. Index values came from Hilsenhoff (1987, 1988). A family level HBI was calculated as:

$$HBI = \sum (n_i \, t_i \, / \, N) \qquad (5)$$

where n_i is the number of individuals of a taxon, t_i is the tolerance value of that taxon, and N is the total number of organisms in a sample. Samples with HBI values of 0-2 are considered clean, 2-4 slightly enriched, 4-7 enriched, and 7-10 polluted. Rather than using mean HBI values for a sample, taxon HBI values can also be used to determine the number of pollution intolerant and tolerant taxa occurring at a site. Taxa with HBI values of 0-2 are considered intolerant clean water taxa and taxa with HBI values of 9-10 are considered pollution tolerant taxa.

USFS Community tolerant quotient/biotic condition index - This index has been widely used by the USFS and BLM throughout the western United States. Taxa are assigned a tolerant quotient (TQ) from 2-taxa found only in high quality unpolluted water, to 108 - taxa found in severely polluted waters. TQ values were developed by Winget and Mangum (1979). The community tolerance quotient (CTQa) was calculated as:

$$CTQa = \sum (TQ \ / \ S) \tag{6}$$

where TQ is the tolerance quotient of that taxon and S is the number of taxa in the sample. The dominance weighted community tolerance quotient (CTQd) was calculated as:

$$CTQd = \sum (n_i \ TQ \ / \ N) \tag{7}$$

where TQ is the tolerance quotient of that taxon, n_i is the number of individuals of a taxon, and N is the total number of organisms in the sample. If data on total alkalinity, sulfate, substrate size, and stream gradient was collected, the predicted community tolerance quotient (CTQp) was calculated. This is a prediction of the unimpacted benthic aquatic macroinvertebrate community structure based on these physical and chemical variables. If the CTQp was calculated, the biotic condition index (BCI) was calculated as:

$$BCI = \frac{CTQp}{CTQd} \times 100 \tag{8}$$

Invertebrate samples with BCIs >90 are considered to come from streams in excellent condition, 80-90 good condition, 72-79 fair condition, and <72 poor condition.

Functional feeding group measures - A useful classification scheme for aquatic macroinvertebrates is to categorize them by feeding acquisition mechanisms. Categories are based on food particle size and food location, e.g., suspended in the water column, deposited in sediments, leaf litter, or live prey. This classification system reflects the major source of the resource, either within the stream itself or from riparian or upland areas and the primary location, either erosional or depositional habitats. The character of a stream can be determined by evaluating the relative proportions of functional groups.

Shredders - Shredders use both living vascular hydrophytes and decomposing vascular plant tissue - coarse particulate organic matter (CPOM). Shredders are sensitive to changes in riparian vegetation. Shredders can be good indicators of toxicants that adhere to organic matter.

Scrapers - Scrapers feed on periphyton - attached algae and associated material. Scraper populations increase with increasing abundance of diatoms and can decrease as filamentous algae, mosses, and vascular plants increase. Scrapers decrease in relative abundance in response to sedimentation and organic pollution.

Collector-filterers - Collector-filterers feed on suspended fine particulate organic matter (FPOM). Collector-gatherers are sensitive to toxicants in the water column and deposited in sediments.

Collector-gatherers - Collector-gatherers feed on deposited fine particulate organic matter. Collector-gatherers are sensitive to deposited toxicants.

Predators - Predators feed on living animal tissue.

Unknown feeding group - This category includes taxa that are highly variable, parasites, and those that for which the primary feeding mode is currently unknown.

Other measures - The number of long-lived taxa and the number of "clinger" taxa have been found by Karr and Chu (1998) to respond negatively to human disturbance. Clinger taxa were determined using information in Merritt and Cummins (1996). These taxa typically cling to the tops of rocks and are thought to be reduced by sedimentation or abundant algal growths. Long-lived taxa are those taxa that typically have 2-3 year life cycles. Disturbances and water quality and habitat impairment typically reduces the number of long-lived taxa.

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Results

Abundance data are reported as the estimated number of individuals per square meter for quantitative samples and the estimated number of individuals collected for qualitative samples. Taxa richness data are the number per sample. NC = Not calculated. * = unable to calculate. EPT = totals for the insect orders, Ephemeroptera, Plecoptera, Trichoptera. In station descriptor, QL identifies qualitative samples.

General Assemblage Measures

Station	Date	Sample ID	Total abundance	EPT abundance	# of families	Dominant family	Dom. Family abundance	Dom. Family % contribution
PNFWALKER1	11/03/2002	119869	4194	1366	23	Chironomidae	1304	31.09
PNFWALKER2	11/03/2002	119870	198	41	12	Chironomidae	113	57.07
PNFWALKER3	11/04/2002	119871	5463	1941	16	Chironomidae	3036	55.57
PNFWALKER4	11/06/2002	119872	5591	2867	18	Chironomidae	2281	40.80
PNFWALKER5	11/09/2002	119873	5241	4407	20	Hydropsychidae	1360	25.95
PNFWALKER6	11/11/2002	119874	2054	1633	22	Heptageniidae	432	21.03
PNFWALKER7	11/15/2002	119875	1512	1072	17	Hydropsychidae	563	37.24
PNFWALKER8	11/14/2002	119876	2723	2123	19	Hydropsychidae	1107	40.65
Mean			3372	1931	18		1275	37.80

Diversity Indices

Station	Date	Sample ID	Total taxa richness	EPT taxa richness	Shannon diversity	Simpson diversity	Evenness
PNFWALKER1	11/03/2002	119869	35	16	2.571	0.136	0.525
PNFWALKER2	11/03/2002	119870	15	10	1.593	0.353	0.469
PNFWALKER3	11/04/2002	119871	26	15	2.197	0.171	0.604
PNFWALKER4	11/06/2002	119872	35	22	2.432	0.157	0.519
PNFWALKER5	11/09/2002	119873	41	25	2.766	0.114	0.520
PNFWALKER6	11/11/2002	119874	42	29	3.059	0.067	0.690
PNFWALKER7	11/15/2002	119875	31	16	2.720	0.096	0.664
PNFWALKER8	11/14/2002	119876	34	22	2.723	0.106	0.595
Mean			32.4	19.4	2.508	0.150	0.573

Biotic Indices

					United States Forest Service Biotic Condition Index				
Station	Date	Sample ID	Hilsenhoff Biotic Index	Indication	CTQp	CTQa	CTQd	BCI	Indication
PNFWALKER1	11/03/2002	119869	3.84	Slight organic enrichment	NC	69	70		
PNFWALKER2	11/03/2002	119870	4.71	Moderate organic enrichment	50	66	81	62	Poor
PNFWALKER3	11/04/2002	119871	4.37	Moderate organic enrichment	50	71	77	65	Poor
PNFWALKER4	11/06/2002	119872	3.59	Slight organic enrichment	NC	58	63		
PNFWALKER5	11/09/2002	119873	2.68	Slight organic enrichment	66	55	54	122	Excellent
PNFWALKER6	11/11/2002	119874	2.90	Slight organic enrichment	50	51	53	94	Excellent
PNFWALKER7	11/15/2002	119875	3.44	Slight organic enrichment	66	78	82	80	Good
PNFWALKER8	11/14/2002	119876	3.72	Slight organic enrichment	50	73	75	67	Poor
Mean			3.66		55	65	69		

Taxa richness and relative abundance values with respect to tolerance or intolerance to pollution were based on the Hilsenhoff Biotic Index (HBI). Intolerant taxa are those taxa given a HBI score of 0, 1, or 2. Tolerant taxa are those taxa given a HBI score of 8, 9, or 10. Abundance data are presented as the estimated number per square meter for quantitative samples and the estimated number of individuals collected for qualitative samples. Taxa richness data are presented as the number of taxa per sample. Numbers in parentheses are percentages of the total. In station descriptor, QL = qualitative sample.

			Intolerant		Tolerant	
Station	Date	Sample ID	Richness	Abundance	Richness	Abundance
PNFWALKER1	11/03/2002	119869	10 (29)	734 (18)	1 (3)	88 (2)
PNFWALKER2	11/03/2002	119870	6 (40)	24 (12)	0 (0)	0 (0)
PNFWALKER3	11/04/2002	119871	8 (31)	418 (8)	1 (4)	192 (4)
PNFWALKER4	11/06/2002	119872	15 (43)	937 (17)	1 (3)	43 (1)
PNFWALKER5	11/09/2002	119873	19 (46)	2181 (42)	0 (0)	0 (0)
PNFWALKER6	11/11/2002	119874	21 (50)	933 (45)	1 (2)	200 (10)
PNFWALKER7	11/15/2002	119875	8 (26)	291 (19)	3 (10)	29 (2)
PNFWALKER8	11/14/2002	119876	10 (29)	362 (13)	2 (6)	64 (2)
Mean			12	735	1	77

Functional feeding groups

Taxa richness by functional feeding group.
Data are presented as the number of taxa collected. Numbers in parentheses are percentages of the total. In station descriptor, QL = qualitative sample.

Station	Date	Sample ID	Shredders	Scrapers	Collector filterers	Collector gatherers	Predators	Unknown
PNFWALKER1	11/03/2002	119869	8 (23)	4 (11)	2 (6)	7 (20)	10 (29)	4 (11)
PNFWALKER2	11/03/2002	119870	2 (13)	0 (0)	1 (7)	4 (27)	6 (40)	2 (13)
PNFWALKER3	11/04/2002	119871	4 (15)	0 (0)	2 (8)	5 (19)	12 (46)	3 (12)
PNFWALKER4	11/06/2002	119872	3 (9)	4 (11)	3 (9)	7 (20)	16 (46)	2 (6)
PNFWALKER5	11/09/2002	119873	4 (10)	5 (12)	2 (5)	8 (20)	17 (41)	5 (12)
PNFWALKER6	11/11/2002	119874	4 (10)	7 (17)	2 (5)	10 (24)	14 (33)	5 (12)
PNFWALKER7	11/15/2002	119875	2 (6)	4 (13)	5 (16)	12 (39)	6 (19)	2 (6)
PNFWALKER8	11/14/2002	119876	4 (12)	4 (12)	4 (12)	13 (38)	7 (21)	2 (6)
Mean			4 (12)	4 (11)	3 (8)	8 (25)	11 (34)	3 (10)

Invertebrate abundance by functional feeding group. Data are presented as the estimated number of individuals per square meter for quantitative samples and the estimated number of individuals collected for qualitative samples. Numbers in parentheses are percentages of the total. In station descriptor, QL = qualitative sample.

Station	Date	Sample ID	Shredders	Scrapers	Collector filterers	Collector gatherers	Predators	Unknown
PNFWALKER1	11/03/2002	119869	592 (14)	1032 (25)	264 (6)	1816 (43)	426 (10)	64 (2)
PNFWALKER2	11/03/2002	119870	11 (6)	0 (0)	1 (1)	117 (59)	39 (20)	30 (15)
PNFWALKER3	11/04/2002	119871	650 (12)	0 (0)	85 (2)	2705 (50)	1233 (23)	789 (14)
PNFWALKER4	11/06/2002	119872	288 (5)	128 (2)	417 (7)	2624 (47)	1162 (21)	972 (17)
PNFWALKER5	11/09/2002	119873	1144 (22)	544 (10)	1376 (26)	944 (18)	932 (18)	301 (6)
PNFWALKER6	11/11/2002	119874	324 (16)	566 (28)	136 (7)	464 (23)	443 (22)	121 (6)
PNFWALKER7	11/15/2002	119875	173 (11)	133 (9)	686 (45)	379 (25)	77 (5)	64 (4)
PNFWALKER8	11/14/2002	119876	144 (5)	197 (7)	1134 (42)	1013 (37)	96 (4)	139 (5)
Mean			416 (12)	325 (10)	512 (15)	1258 (37)	551 (16)	310 (9)

The 10 metrics thought to be most responsive to human-induced disturbance (Karr and Chu 1998).

Station	Date	Sample ID	Total taxa	Ephemeroptera taxa	Plecoptera taxa	Trichoptera taxa	Long-lived taxa	Intolerant taxa	% tolerants	Clinger taxa	% contribution dominant taxon	% predators
PNFWALKER1	11/03/2002	119869	35	5	6	5	8	10	2.1	14	27.7	10.2
PNFWALKER2	11/03/2002	119870	15	3	3	4	0	6	0.0	6	57.1	19.7
PNFWALKER3	11/04/2002	119871	26	1	6	8	2	8	3.5	9	33.6	22.6
PNFWALKER4	11/06/2002	119872	35	6	9	7	4	15	0.8	17	32.6	20.8
PNFWALKER5	11/09/2002	119873	41	7	10	8	7	19	0.0	17	25.9	17.8
PNFWALKER6	11/11/2002	119874	42	11	11	7	5	21	9.7	18	14.0	21.6
PNFWALKER7	11/15/2002	119875	31	6	4	6	5	8	1.9	16	21.7	5.1
PNFWALKER8	11/14/2002	119876	34	10	5	7	6	10	2.4	16	25.1	3.5
Mean			32	6	7	7	5	12	2.3	14	28.1	16.3

List of taxa collected in 8 samples at sites listed in Table 1. Samples were collected between 3 November 2002 and 15 November 2002. Abundance data are presented as the mean number of individuals among all samples.

Order	Family	Subfamily/Genus/species	Average abundance
Phylum: Arthropoda			
Class: Arachnida			
Trombidiformes			35.58
Class: Insecta			
Coleoptera	Elmidae		33.33
Coleoptera	Elmidae	Ampumixis	3.00
Coleoptera	Elmidae	Cleptelmis	5.00
Coleoptera	Elmidae	Lara	1.00
Coleoptera	Elmidae	Narpus	1.00
Coleoptera	Elmidae	Optioservus	163.50
Coleoptera	Elmidae	Zaitzevia	11.67
Coleoptera	Psephenidae	Eubrianax	14.00
Diptera	Ceratopogonidae	Culicoides	5.33
Diptera	Ceratopogonidae	Probezzia	17.16
Diptera	Chironomidae		57.14
Diptera	Chironomidae	Chironominae	74.32
Diptera	Chironomidae	Orthocladiinae	705.72
Diptera	Chironomidae	Tanypodinae	137.10
Diptera	Empididae	Chelifera	9.92
Diptera	Empididae	Hemerodromia	1.67
Diptera	Simuliidae		1.33
Diptera	Simuliidae	Prosimulium	1.00
Diptera	Simuliidae	Simulium	20.67
Diptera	Tabanidae	Tabanus	0.75
Diptera	Tipulidae		2.00
Diptera	Tipulidae	Antocha	97.58
Diptera	Tipulidae	Dicranota	8.46
Diptera	Tipulidae	Hexatoma	4.63
Diptera	Tipulidae	Tipula	1.33
Ephemeroptera	Ameletidae	Ameletus	3.00
Ephemeroptera	Baetidae		20.58
Ephemeroptera	Baetidae	Acentrella	0.67
Ephemeroptera	Baetidae	Baetis	90.15
Ephemeroptera	Baetidae	Diphetor hageni	2.00
Ephemeroptera	Ephemerellidae		67.29
Ephemeroptera	Ephemerellidae	Drunella doddsi	15.96
Ephemeroptera	Ephemerellidae	Drunella grandis	0.63
Ephemeroptera	Ephemerellidae	Drunella spinifera	4.83
Ephemeroptera	Heptageniidae		42.50
Ephemeroptera	Heptageniidae	Cinygmula	29.00
Ephemeroptera	Heptageniidae	Epeorus	57.00
Ephemeroptera	Heptageniidae	Ironodes	41.50
Ephemeroptera	Heptageniidae	Rhithrogena	6.00
Ephemeroptera	Leptohyphidae	Tricorythodes	3.33
Ephemeroptera	Leptophlebiidae		19.50
Megaloptera			2.00
Megaloptera	Corydalidae	Orohermes crepusculus	9.21
Megaloptera	Sialidae	Sialis occidens	3.58
Odonata	Coenagrionidae		2.33
Odonata	Gomphidae		2.00
Odonata	Gomphidae	Ophiogomphus	0.38
Plecoptera			266.39
Plecoptera	Capniidae		29.33
Plecoptera	Chloroperlidae	Sweltsa	92.64
Plecoptera	Leuctridae		0.50
Plecoptera	Nemouridae		6.00
Plecoptera	Nemouridae	Malenka	0.50
Plecoptera	Nemouridae	Zapada	41.45
Plecoptera	Peltoperlidae	Yoraperla	2.00
Plecoptera	Perlidae		58.33
Plecoptera	Perlidae	Calineuria	32.42
Plecoptera	Perlidae	Hesperoperla pacifica	1.25
Plecoptera	Perlodidae		26.95
Plecoptera	Perlodidae	Isoperla	36.32
Plecoptera	Perlodidae	Oroperla	1.88

Taxonomic list, continued.

Order	Family	Subfamily/Genus/species	Average abundance
Plecoptera	Perlodidae	Perlinodes	4.63
Plecoptera	Perlodidae	Skwala	5.37
Plecoptera	Pteronarcyidae		1.00
Trichoptera			4.00
Trichoptera	Brachycentridae	Amiocentrus	18.12
Trichoptera	Brachycentridae	Micrasema	179.33
Trichoptera	Glossosomatidae		8.67
Trichoptera	Glossosomatidae	Glossosoma	5.96
Trichoptera	Hydropsychidae		30.00
Trichoptera	Hydropsychidae	Cheumatopsyche	60.42
Trichoptera	Hydropsychidae	Hydropsyche	397.93
Trichoptera	Hydroptilidae	Hydroptila	103.86
Trichoptera	Hydroptilidae	Oxyethira	0.67
Trichoptera	Lepidostomatidae	Lepidostoma	29.33
Trichoptera	Polycentropodidae	Polycentropus	2.67
Trichoptera	Rhyacophilidae		3.00
Trichoptera	Rhyacophilidae	Rhyacophila	38.53
Trichoptera	Rhyacophilidae	Rhyacophila betteni group	8.00
Trichoptera	Rhyacophilidae	Rhyacophila brunnea group	11.96
Trichoptera	Sericostomatidae	Gumaga	18.00
Class: Malacostraca			
Amphipoda	Hyalellidae	Hyalella azteca	0.33
Phylum: Mollusca			
Class: Bivalvia			
Veneroida	Pisidiidae	Pisidium	1.00
Class: Gastropoda			1.00
Basommatophora	Planorbidae		2.00
Phylum: Platyhelminthes			
Class: Turbellaria			2.67

A total of 87 taxa were collected in 8 samples.			3371.98

BIOASSESSMENT 2002

PERIPHYTON (ALGAE) COMMUNITY REPORT

BIOLOGICAL INTEGRITY OF LITTLE GRIZZLY CREEK AND INDIAN CREEK BASED ON THE COMPOSITION AND STRUCTURE OF THE BENTHIC ALGAE COMMUNITY

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Summary

In November 2002, periphyton samples were collected from 8 sites on Little Grizzly Creek and Indian Creek on the Plumas National Forest as part of the Walker Mine Tailings Project. The samples were collected following the U.S. Forest Service's Pacific Southwest Region Stream Bioassessment Protocols and processed and analyzed using standard methods for periphyton. The results were evaluated using modified USEPA rapid bioassessment protocols for wadeable streams. The purpose of this report is to determine the degree of impairment at each of the sample sites and to ascertain the likely cause or causes of impairment at sites where impairment is evident.

A combination of acid waters and elevated concentrations of heavy metals appeared to cause at least moderate impairment to aquatic life at sites 2 and 5 on Little Grizzly Creek. Both sites had low diatom species richness and diversity and were dominated by *Achnanthidium minutissimum*, a species known to be tolerant of acid mine drainage. Although all of the sites supported at least a few teratological diatom cells, site 2 supported the largest percentage.

Loading by both organic and inorganic nutrients appeared to cause moderate impairment at site 3 on Little Grizzly Creek. Two species of diatoms dominated at this site: *Rhopalodia gibba* and *Nitzschia palea*. The first is a eutraphentic nitrogen autotroph that requires large amounts of inorganic nutrients. The second is an obligate nitrogen heterotroph.

Excessive sedimentation for a mountain stream caused at least moderate impairment at sites 1 and 4 on Little Grizzly Creek. Recovery from all perturbations on Little Grizzly Creek was nearly complete at site 6, where diatom metrics demonstrated good biological integrity with only minor impairment from sedimentation and heavy metals.

Both sites on Indian Creek had very similar algal floras, indicating that any pollutants discharged by Little Grizzly Creek had little or no effect. The dominant diatom species in Indian Creek was *Staurosira construens*, a sensitive, alkaliphilous species that indicates low levels of organic matter, moderate concentrations of inorganic nutrients, high concentrations of dissolved oxygen, and stable flows with low disturbance. Indian Creek had good biological integrity with only minor impairment from sedimentation.

All of the major diatom species in Little Grizzly Creek and Indian Creek are cosmopolitan and found in disturbed watersheds worldwide. These streams supported few unknown diatom species and no recognized endemic species, which one would expect to find in more pristine habitats.

Biocriteria used to judge impairment in this report were derived from samples collected in summer from the Rocky Mountain ecoregions of western Montana. Because these samples were collected from Sierra Nevada streams in the fall, estimates of use impairment contained in this report may be inaccurate.

Introduction

This report evaluates the biological integrity[1] of aquatic communities at selected sites on Little Grizzly Creek and Indian Creek on the Plumas National Forest, California. The purpose of this report is to provide information that will help the Plumas National Forest and the State of California identify which of these stream sites is impaired, estimate the level of impairment or support of aquatic life uses at each site, and ascertain the likely cause or causes of impairment at each impaired site.

Evaluation of aquatic life use support in this report is based on the species composition and structure of periphyton (benthic algae, phytobenthos) communities at 8 sites that were sampled in November 2002. Periphyton is a diverse assortment of simple photosynthetic organisms called algae that live attached to or in close proximity of the stream bottom. Some algae form long filaments or large colonies and are conspicuous to the unaided eye. But most, including the ubiquitous diatoms, can be seen and identified only with the aid of a microscope. The periphyton community is a basic biological component of all aquatic ecosystems. Periphyton accounts for much of the primary production and biological diversity in Montana streams (Bahls et al. 1992). Plafkin et al. (1989) and Barbour et al. (1999) list several advantages of using periphyton in biological assessments.

Project Area and Sampling Sites

The project area is located within the Sierra Nevada Ecoregion of the United States (Omernik 1986) in Plumas County, California. Vegetation is mainly mixed conifer forest (Bailey 1995). The main land uses in the Little Grizzly and Indian Creek watersheds are grazing, logging, mining, agriculture, and recreation. Little Grizzly Creek is a tributary of Indian Creek, which is a tributary of the East Branch North Fork Feather River (HUC 18020122).

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[1] *Biological integrity* is defined as "the ability of an aquatic ecosystem to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitats within a region" (Karr and Dudley 1981).

Periphyton samples were collected at 6 sites on Little Grizzly Creek and 2 sites on Indian Creek, one above and one below the confluence with Little Grizzly Creek (Table 1). Elevations at the sample sites range from 5800 feet at the uppermost site on Little Grizzly Creek to 3670 feet at the lower site on Indian Creek. Little Grizzly Creek is a 3rd order stream and Indian Creek is a 4th order stream (Strahler 1957). Based on pebble count data, the modal category for substrate particle size at each site ranged from 64 mm to >180 mm diameter (source: The Bug Lab's Aquatic Macroinvertebrate Stream Health Assessment Information). Values for selected environmental variables that were measured or estimated at the time periphyton samples were collected are presented in Table 2.

Methods

Periphyton samples were collected using the USDA Forest Service's Pacific Southwest Region Stream Bioassessment Protocols (Version 2001-1). For each site, a reach of not less than 500 m was established. Within this reach, four fast-water (riffle) habitats were selected in sequence, beginning near the downstream end of the reach. Two pieces of substrate in the 15-20 cm diameter size class were sampled in each riffle, for a total of 8 samples from the reach. Periphyton was brushed or scraped from a delimited area on the surface of each piece of substrate and composited into a single sample bottle. The total substrate area represented by each composite periphyton sample was 96.0 cm^2. Samples were preserved with 10% formalin.

The samples were examined to estimate the relative abundance of cells and ordinal rank by biovolume of diatoms and genera of soft (non-diatom) algae according to the method described in Bahls (1993). Soft algae were identified using Smith (1950), Prescott (1962, 1978), and John et al. (2002). These books also served as references on the ecology of the soft algae, along with Palmer (1969, 1977).

After the identification of soft algae, the raw periphyton samples were cleaned of organic matter using sulfuric acid, potassium dichromate, and hydrogen peroxide. Then permanent diatom slides were prepared using Naphrax, a high refractive index mounting medium, following

Standard Methods for the Examination of Water and Wastewater (APHA 1998). Between 419 and 464 diatom cells (838 to 928 valves) were counted at random and identified to species. The following were the main taxonomic references for the diatoms: Krammer and Lange-Bertalot 1986, 1988, 1991a, 1991b. Diatom naming conventions followed those adopted by the Academy of Natural Sciences for USGS NAWQA samples (Morales and Potapova 2000). Van Dam et al. (1994) was the main ecological reference for the diatoms.

The diatom proportional counts were used to generate an array of diatom association metrics. A metric is a characteristic of the biota that changes in some predictable way with increased human influence (Barbour et al. 1999). Diatoms are particularly useful in generating metrics because there is a wealth of information available in the literature regarding the pollution tolerances and water quality preferences of common diatom species (e.g., Lowe 1974, Beaver 1981, Van Dam et al. 1994, Lange-Bertalot 1996).

Values for selected metrics were compared to biocriteria (numeric thresholds) developed for streams in the Rocky Mountain ecoregions of western Montana (Table 3). These criteria are based on metric values measured in least-impaired reference streams (Bahls et al. 1992) and on metric values measured in streams that are impaired by various sources and causes of pollution (Bahls 1993). The biocriteria in Table 3 were established for samples collected during the summer field season (June 21-September 21).

The criteria in Table 3 distinguish among four levels of stress or impairment and three levels of aquatic life use support: (1) no impairment or only minor impairment (full support); (2) moderate impairment (partial support); and (3) severe impairment (nonsupport). These impairment levels correspond to excellent, good, fair, and poor biological integrity, respectively. In cold, high-gradient mountain streams, natural stressors will sometimes mimic the effects of man-caused impairment on some metric values. Because the samples were collected outside the Rocky Mountain ecoregions and outside the summer field season, the criteria and impairment levels in Table 3 may not be appropriate for evaluating periphyton data generated from samples collected from Sierra Nevada streams in the fall.

Quality Assurance

Several steps were taken to assure that the study results are accurate and reproducible.

Upon receipt of the samples, station and sample attribute data were recorded in the Montana Diatom Database and the samples were assigned a unique number, e.g., 2580-01. The first part of this number (2580) designates the sampling site (Little Grizzly Creek Site 1) and the second part (01) designates the number of periphyton samples that that have been collected at this site for which data have been entered into the Montana Diatom Database.

Sample observations and analyses of soft (non-diatom) algae were recorded in a lab notebook along with information on the sample label. A portion of the raw sample was used to make duplicate diatom slides. The slide used for the diatom proportional count will be deposited in the Montana Diatom Collection at the University of Montana Herbarium in Missoula. The duplicate slide will be retained by *Hannaea* in Helena. Diatom proportional counts have been entered into the Montana Diatom Database.

Results and Discussion

Periphyton results are presented in Tables 4 and 5 and Figures 1, 2, and 3, which are located near the end of this report following the references section. The Appendix contains a series of diatom reports, one for each sample. Each diatom report contains an alphabetical list of diatom species and their percent abundances, and values for 65 different diatom metrics and ecological attributes.

Sample Notes

Little Grizzly Creek. At site 2, particulate organic matter (POM) was common. Most of the cyanobacteria at this site were embedded in clumps of POM. The average size of diatoms here was smaller than at site 1 and *Nitzschia* spp. were common. At site 3, *Rhopalodia gibba* was a dominant diatom and several abnormal valves of this species were observed.

Indian Creek. The sample from site 7 had a rusty cast and the sample from site 8 had a brownish cast.

Non-Diatom Algae

Little Grizzly Creek. The periphyton community of Little Grizzly Creek was dominated by diatoms and genera of cyanobacteria (Table 4). Some of these blue-green algae (*Anabaena, Nostoc, Tolypothrix)* have heterocysts and are capable of fixing atmospheric nitrogen, indicating that nitrogen may be the limiting nutrient in this stream. Green algae (Chlorophyta) were encountered only occasionally at sites 1 and 2. *Tribonema,* a filamentous xanthophyte, was recorded at sites 3 and 5, where it was found only occasionally. The number of non-diatom genera decreased by more than half (from 12 to 5) between sites 2 and 3, and decreased by more than half again (from 5 to 2) between sites 5 and 6 (Table 4).

Indian Creek. Diatoms and cyanobacteria also dominated the periphyton community in Indian Creek, although green algae were somewhat more common here than they were in Little Grizzly Creek (Table 4). The xanthophyte *Tribonema* was common in the sample from the upstream site, but was not observed in the sample from the downstream site. The cyanophyte *Tolypothrix,* also a clean-water indicator, was common at the downstream site but was not observed at the upstream site. Most of the other common genera were present at both sites. The number of non-diatom algal genera at the two sites on Indian Creek was nearly identical.

Diatoms

Of the 17 major diatom species found in Little Grizzly Creek and Indian Creek, 10 are sensitive to organic pollution, 6 are somewhat tolerant of organic pollution, and 1 is most tolerant of organic pollution (Table 5). The only major species that is most tolerant of organic pollution is *Nitzschia palea,* which was a dominant species at site 3. This species is an obligate nitrogen heterotroph and requires large concentrations of organic nitrogen for optimum growth and population development.

Another major species was *Achnanthidium minutissimum* (synonym *Achnanthes minutissima*), which was the dominant species at sites 2 and 5. This is a cosmopolitan species with very small cells that attach to hard substrates by a short stalk. Although sensitive to organic pollution and an indicator of low concentrations of inorganic phosphorus, *A. minutissimum* is tolerant of heavy metals and a wide range of pH values. It is one of the most common diatoms in streams receiving acid mine drainage. Because of its attached growth form and low profile, it is also resistant to physical and biological disturbance in the form of scour and grazing by macroinvertebrates.

Rhopalodia gibba, a co-dominant diatom species at site 3, is a nitrogen autotroph and a eutraphentic species. It may also, under the right conditions, harbor nitrogen-fixing cyanobacteria as endosymbionts, however none were observed in the sample from this site.

All of the major diatom species in Table 5 are cosmopolitan in distribution and found in disturbed watersheds worldwide. Little Grizzly Creek and Indian Creek supported few unknown diatom species and no recognized endemic species, which would be expected in more pristine habitats.

All of the sites in both Little Grizzly Creek and Indian Creek supported at least a few teratological (physically abnormal) diatom cells. (Grizzly Creek site 2 supported the largest percentage.) This may indicate that there are low background concentrations of heavy metals in these streams due to natural mineralization in the watershed.

Little Grizzly Creek. A large percentage of motile diatoms indicate that the most upstream site on Little Grizzly Creek (site 1) may be impaired by sedimentation (Table 5). On the other hand, the most abundant diatom species at this site (*Nitzschia fonticola*) is sensitive to organic pollution. A few abnormal diatom valves at site 1 may indicate low background concentrations of heavy metals, but these may be due to natural mineralization in the watershed. All other metrics examined for this site indicate no impairment, excellent biological integrity, and full support of aquatic life uses when compared to periphyton communities in least-impaired reference sites in the Rocky Mountain ecoregions of Montana in summer.

A dramatic change in the diatom assemblage occurred between sites 1 and 2, which shared less than 25% of their floras (Table 5). *Nitzschia fonticola* almost disappeared and *Achnanthidium minutissimum* became the dominant species at site 2, accounting for more than three-quarters of the diatom cells counted. The number of diatom species declined by more than half and Shannon species diversity declined to slightly more than one-half of its upstream value. Site 2 supported the largest percentage of teratological diatom cells of all sites, indicating that this site likely had had the largest concentrations of heavy metals. The low species diversity and high level of disturbance at this site was probably caused by acidity and/or chemical toxicity.

Another large change occurred between sites 2 and 3, where *Rhopalodia gibba* and *Nitzschia palea* were the dominant species (Table 5). These species indicate a significant increase in nutrient enrichment, both organic and inorganic. The pollution index at site 3 dipped into the range of moderate impairment and fair biological integrity for Rocky Mountain streams in summer. Species richness at sites 2 and 3 were comparably low and the sites shared only about one-third of their diatom floras.

A smaller change occurred between sites 3 and 4, which shared slightly less than half of their diatom floras (Table 5). However, site 4 had the lowest species richness and the largest sedimentation index of all the sites. The dominant diatom species here were *Navicula cryptotenella* and *Nitzschia dissipata*. Both are motile, cosmopolitan species with broad ecological amplitudes. A small increase in the pollution index here indicates that the stream had begun to assimilate and recover from the nutrient inputs upstream.

A major change occurred again between sites 4 and 5, which shared about one-third of their diatom floras (Table 5). The diatom assemblage at site 5 was similar to site 2 in that it was dominated by *Achnanthidium minutissimum*. The large percentage of this diatom indicates moderate disturbance, perhaps from acid waters or metals toxicity, or both. Diatom species richness and diversity were low at site 5.

A significant amount of recovery occurred between sites 5 and 6 (Table 5). Diatom metrics at site 6 indicated good biological integrity and full support of aquatic life uses with only minor impairment from sedimentation and heavy metals (% abnormal cells). Normal values for the pollution index and the disturbance index indicate that the stream had recovered here from nutrient and acid mine inputs upstream. *Cocconeis placentula*, the dominant species at this site, is an alkaliphilous and eutraphentic diatom that attaches by its concave valve face to hard substrates (rocks). It is sensitive to excessive sedimentation, organic loading, and heavy metals.

Indian Creek. Both sites on Indian Creek had good biological integrity. Diatom metrics indicated full support of aquatic life uses with only minor impairment from sedimentation and heavy metals. The two sites shared over 70% of their diatom floras, indicating that Little Grizzly Creek had little or no effect on diatom composition at the downstream site. Without intervening tributaries or pollution sources, one may expect two adjacent reaches on the same stream to have at least 60% of their diatom floras in common (Bahls 1993). The dominant diatom species in Indian Creek was *Staurosira construens,* a sensitive, alkaliphilous species that indicates low levels of organic matter, moderate concentrations of inorganic nutrients, high concentrations of dissolved oxygen, and stable flows with low disturbance.

Diagnostic Metrics

In addition to the metrics presented in Table 5, several diagnostic ecological metrics were extracted from the diatom reports in the Appendix and examined to determine the causes of changes observed at each station. Three sets of paired metrics were used to evaluate changes due to (1) pH, (2) nitrogen enrichment, and (3) loading by organic and inorganic nutrients.

pH. Alkaliphilous diatoms (pH Group 4, Van Dam et al. 1994) occur mainly at pH >7. Alkalibiontic diatoms (pH Group 5, Van Dam et al. 1994) occur exclusively at pH >7. Circumneutral diatoms (pH Group 3, Van Dam et al. 1994) occur mainly at pH values about 7. A shift from a preponderance of alkaliphilous and alkalibiontic diatoms to circumneutral diatoms indicates a decrease in pH.

The majority of diatoms in Little Grizzly Creek and Indian Creek were alkaliphilous and alkalibiontic at all sites except 2 and 5 (Figure 1). These two stations were dominated by *Achnanthidium minutissimum*, a species that prefers circumneutral pH values. This shift indicates a drop in pH, perhaps associated with acid mine drainage. Field pH measurements at the time of periphyton sampling do not indicate significantly lower pH values at sites 2 and 5 (Table 2). Nevertheless, the diatom assemblage may be a better indicator of short- to mid-term pH values at these sites than an instantaneous field measurement.

Nitrogen Metabolism. Nitrogen heterotrophs (Nitrogen Metabolism Groups 3 and 4, Van Dam et al. 1994) need elevated concentrations of organically bound nitrogen. As concentrations of organic nitrogen increase due to human disturbance, the percentage of nitrogen heterotrophs in the diatom association will also increase. Diatoms in the family Epithemiaceae (*Epithemia* spp., *Rhopalodia* spp., and *Denticula* spp.) commonly harbor cells of the coccoid blue-green alga *Synechococcus* as endosymbionts in nitrogen-poor lakes, streams, and marshes (Burkholder 1996). This association has been shown to fix nitrogen (Stewart et al. 1980). Thus, when concentrations of inorganic nitrogen are low, one may expect the percentage of diatoms in the family Epithemiaceae to increase. *Rhopalodia gibba* and *Epithemia sorex* are the most common representatives of this family in Little Grizzly Creek and Indian Creek. Both are nitrogen autotrophs and eutraphentic species, and neither one was observed to harbor endosymbiotic cyanobacteria in these streams.

Nitrogen heterotrophs achieved their maximum abundance at site 3 (Figure 2), where *Nitzschia palea* was one of the two dominant species. This indicates that site 3 received the largest loads of organic nitrogen of all the sites. Site 3 also supported the highest percentage of diatoms in the family Epithemiaceae. The most abundant of these species was *Rhopalodia gibba*, a nitrogen autotroph and eutraphentic diatom. This indicates that supplies of inorganic nitrogen were also highest at site 3.

Organic and Inorganic Nutrients. Polysaprobous diatoms (Saprobity Group 5, Van Dam et al. 1994) characterize waters with heavy loads of organic matter and where oxygen is usually absent or present in small concentrations. The percentage of polysaprobous diatoms will increase as organic loads from human disturbance (e.g., from agriculture and wastewater discharges) increase. *Nitzschia palea* is the most common polysaprobous diatom in these samples. Eutraphentic and hypereutraphentic diatoms (Trophic State Groups 5 and 6, Van Dam et al. 1994) indicate elevated concentrations of nutrients that are important for diatom growth: nitrogen, phosphorus, inorganic carbon, and silica. As concentrations of these nutrients increase due to human disturbance, the percentage of eutraphentic and hypereutraphentic diatoms will also increase.

The percentage of polysaprobous diatoms (mainly *Nitzschia palea*) peaked at site 3 (Figure 3). Therefore, this site most likely receives the largest load of organic nutrients. Conspicuous peaks of eutraphentic diatoms (mainly *Rhopalodia gibba* and *Cocconeis placentula*) plus hypereutraphentic diatoms (mainly *Nitzschia palea*) occurred at sites 3 and 6. These sites likely have the highest concentrations of inorganic nutrients. Alternatively, sites 2 and 5 appear to have the smallest concentrations of inorganic nutrients.

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Table 1. Locations and sampling dates for periphyton stations on Little Grizzly Creek and Indian Creek.

Site No.	Waterbody Name	Station ID	UTM Coordinates	Sample Date
1	Little Grizzly Creek	PNFWALKER 1	0699130-4424389	11/3/2002
2	Little Grizzly Creek	PNFWALKER 2	0697614-4425160	11/3/2002
3	Little Grizzly Creek	PNFWALKER 3	0696718-4425710	11/4/2002
4	Little Grizzly Creek	PNFWALKER 4	0695528-4426924	11/6/2002
5	Little Grizzly Creek	PNFWALKER 5	0694859-4429242	11/9/2002
6	Little Grizzly Creek	PNFWALKER 6	0691770-4431715	11/11/2002
7	Indian Creek above L. Grizzly Cr.	PNFWALKER 7	0691079-4434634	11/15/2002
8	Indian Creek below L. Grizzly Cr.	PNFWALKER 8	0689830-4434876	11/14/2002

Table 2. Values for selected environmental variables measured or estimated at periphyton sampling sites on periphyton sampling dates. Source: "The Bug Lab's Aquatic Macroinvertebrate Stream Health Assessment Information"

Variable	Unit	Station							
		1	2	3	4	5	6	7	8
Elevation	feet	5800	5700	5400	5040	4720	4250	3683	3670
Conductivity	micro Siemens	70.1	94.6	101.9	103.0	86.6	93.6	93.7	95.3
Field pH	standard units	7.29	7.59	8.36	8.38	7.92	8.02	7.75	7.76
Air Temperature	°C	12.0	9.0	11.0	9.0	8.0	10.0	10.0	9.0
Water Temperature	°C	2.0	0.9	9.0	2.2	4.5	5.5	5.8	6.5
Stream Gradient	%	1.5	4.3	4.0	5.0	3.0	4.5	1.5	1.5
Average Velocity	ft/sec	1.3	0.54	1.2	1.3	1.8	2.5	1.7	1.3
Overstream Shade	%	48.4	19.4	11.4	19.4	4.9	55.7	0.0	2.3

Table 3. Diatom association metrics used by the State of Montana to evaluate biological integrity in mountain streams: references, range of values, expected response to increasing impairment or natural stress, and criteria for rating levels of biological integrity. The lowest rating for any one metric is the rating for that site.

Biological Integrity/ Impairment or Stress/ Use Support	No. of Species Counted[1]	Diversity Index[2] (Shannon)	Pollution Index[3]	Siltation Index[4]	Disturbance Index[5]	% Dominant Species[6]	% Abnormal Cells[7]
Excellent/None Full Support	>29	>2.99	>2.50	<20.0	<25.0	<25.0	0
Good/Minor Full Support	20-29	2.00-2.99	2.01-2.50	20.0-39.9	25.0-49.9	25.0-49.9	>0.0, <3.0
Fair/Moderate Partial Support	19-10	1.00-1.99	1.50-2.00	40.0-59.9	50.0-74.9	50.0-74.9	3.0-9.9
Poor/Severe Nonsupport	<10	<1.00	<1.50	>59.9	>74.9	>74.9	>9.9
References	Bahls 1979 Bahls 1993	Bahls 1979	Bahls 1993	Bahls 1993	Barbour et al. 1999	Barbour et al. 1999	McFarland et al. 1997
Range of Values	0-100+	0.00-5.00+	1.00-3.00	0.0-90.0+	0.0-100.0	~5.0-100.0	0.0-30.0+
Expected Response	Decrease[8]	Decrease[8]	Decrease	Increase	Increase	Increase	Increase

[1]Based on a proportional count of 400 cells (800 valves)

[2]Base 2 [bits] (Weber 1973)

[3]Composite numeric expression of the pollution tolerances assigned by Lange-Bertalot (1979) to the common diatom species

[4]Sum of the percent abundances of all species in the genera *Navicula, Nitzschia* and *Surirella*

[5]Percent abundance of Achnanthidium minutissimum (synonym: *Achnanthes minutissima*)

[6]Percent abundance of the species with the largest number of cells in the proportional count

[7]Cells with an irregular outline or with abnormal ornamentation, or both

[8]Species richness and diversity may increase somewhat in mountain streams in response to slight to moderate increases in nutrients or sediment

Table 4. Relative abundance of cells and ordinal rank by biovolume of diatoms (Division Bacillariophyta) and genera of non-diatom algae in periphyton samples collected from Little Grizzly Creek and Indian Creek in November 2002: d = dominant; a = abundant; f = frequent; c = common; o = occasional; r = rare.

Taxa	Station							
	1	2	3	4	5	6	7	8
Cyanophyta								
Amphithrix							o/10	r/11
Anabaena	c/4	a/2		o/7			r/13	
Calothrix		f/5	f/4	c/6				
Chroococcus	r/12							
Microcoleus		o/9						
Nostoc		c/6	f/3	f/4	f/2	a/1	f/2	r/10
Oscillatoria	r/11	r/13	c/5	c/5			c/4	c/4
Phormidium		f/4					f/3	
Pleurocapsa	o/7	c/7			o/6			
Rivularia			d/2	a/2	o/4		c/5	o/5
Synechocystis		a/3						
Tolypothrix	f/2			f/3	c/3			c/2
Chlorophyta								
Ankistrodesmus	o/10					r/3	c/7	c/6
Cladophora								r/9
Closterium	c/3							
Cosmarium	o/9	o/10						r/12
Mougeotia	r/13	o/8					o/8	c/3
Oedogonium							r/12	
Pediastrum		r/12						o/7
Scenedesmus	o/8	o/11					o/11	o/8
Stigeoclonium	o/6						o/9	
Zygnema	o/5							
Xanthophyta								
Tribonema			r/6		o/5		c/6	
Bacillariophyta	a/1	a/1	d/1	d/1	d/1	f/2	a/1	d/1
# Non-Diatom Genera	12	12	5	6	5	2	12	11

Table 5. Percent abundance of major diatom species[1] and values of selected diatom association metrics for periphyton samples collected from Little Grizzly Creek and Indian Creek. <u>Underlined values</u> indicate minor impairment; **bold values** indicate moderate impairment; **<u>underlined and bold</u>** values indicate severe impairment; all other values indicate no impairment and full support of aquatic life uses when compared to criteria for mountain streams in Table 2.

Species/Metric	PTC[2]	Station 1	2	3	4	5	6	7	8
Achnanthes lanceolata	2	0.45	0.24	0.54	1.14	8.70	8.31	1.81	2.20
Achnanthidium minutissimum	3	12.53	76.92	17.03	10.83	63.41	5.66	0.91	1.97
Cocconeis placentula	3	9.17	0.12	0.43	0.34	0.60	25.40	5.32	1.85
Epithemia sorex	3	0.56				0.72	0.12	7.47	2.20
Fragilaria atomus	3							1.13	5.32
Navicula cryptotenella	2	8.17	0.12	2.37	27.25	4.65	4.97	2.38	3.70
Navicula tripunctata	3			0.43		0.95	12.70	0.11	0.12
Nitzschia dissipata	3	6.15	0.47	3.88	16.42	5.72	4.73	2.83	7.18
Nitzschia fonticola	3	18.90	0.83		0.23		0.58	9.17	4.98
Nitzschia linearis	2	1.68			6.84	1.91	2.42		0.12
Nitzschia palea	1	0.22	5.44	25.75	7.64	1.31	1.62	1.59	4.05
Nitzschia paleacea	2	7.94				1.79		4.53	4.05
Pseudostaurosira brevistriata	3	1.12						4.42	5.56
Rhopalodia gibba	2	1.23	0.59	29.96	9.35	1.91	4.85		0.81
Staurosira construens	3	3.24			0.46			23.44	28.24
Staurosirella pinnata	3	2.46						4.64	7.29
Synedra ulna	2	1.68	5.33	7.44	4.56	1.79	4.85	0.68	0.35
Number of Species Counted		56	<u>25</u>	<u>26</u>	**19**	<u>22</u>	38	59	53
Shannon Species Diversity		4.38	**1.61**	<u>2.93</u>	3.14	<u>2.24</u>	3.91	4.53	4.23
Pollution Index		2.61	2.72	**1.93**	<u>2.18</u>	2.71	2.59	2.68	2.69
Siltation Index		**51.34**	9.59	<u>38.04</u>	**<u>61.46</u>**	17.16	<u>36.49</u>	<u>34.32</u>	<u>38.19</u>
Disturbance Index		12.53	**<u>76.92</u>**	17.03	10.83	**63.41**	5.66	0.91	1.97
Percent Dominant Species		18.90	**<u>76.92</u>**	<u>29.96</u>	<u>27.25</u>	**63.41**	<u>25.40</u>	23.44	<u>28.24</u>
Percent Abnormal Cells		<u>0.56</u>	<u>2.13</u>	<u>1.08</u>	<u>0.68</u>	<u>0.36</u>	<u>1.15</u>	<u>0.45</u>	<u>0.12</u>
Similarity Index[3]			23.94	32.99	46.34	34.21	37.38		70.72

[1]A major species accounts for 5.0% or more of the cells at one or more stations in a sample set.

[2]Pollution Tolerance Class (Lange-Bertalot 1979): 1 = most tolerant; 2 = tolerant; 3 = sensitive to organic pollution

[3]Percent Community Similarity (Whittaker 1952) when compared to the diatom assemblage at the adjacent upstream station.